



FORMULATING AND EVALUATION OF HERBAL CREAM CONTAINING ANTIMICROBIAL PROPERTIES OF METHANOLIC EXTRACT OF TAMARINDUS INDICA SEEDS

Abhishek Nandu Farkade^{1*}, Gitesh Vinod Vyas², Anand Daulatrao Khendke³

¹*Student of Bachelor in pharmacy, faculty of pharmacy, Dr. Babsaheb Ambedkar Technological University, Raigad, Lonere.*

²*Department of Pharmacology, Faculty of Pharmacology, Dr. Babsaheb Ambedkar Technological University, Raigad, Lonere*

³*Student of Bachelor in pharmacy, faculty of pharmacy, Dr. Babasaheb Ambedkar Technological University, Raigad, Lonere.*

**Corresponding Author*

ABSTRACT

The demand for natural and herbal-based skincare products has been on the rise due to concerns over synthetic ingredients and their potential side effects. This study aimed to formulate and evaluate a herbal cream using the antimicrobial properties of the methanol extract of Tamarindus indica seeds.

The methanol extract of Tamarindus indica seeds was obtained using standard extraction methods and incorporated into a cream base. Various physicochemical properties, including pH, viscosity, spreadability, and stability, were evaluated. The antimicrobial activity of the formulated cream was assessed against a range of pathogenic microorganisms using agar well diffusion and minimum inhibitory concentration (MIC) methods.

The formulated herbal cream demonstrated satisfactory physicochemical properties, with a pH suitable for skin application and good spreadability. The cream exhibited significant antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as fungi. The MIC values indicated the potent antimicrobial efficacy of the Tamarindus indica seed extract in the cream formulation.

KEYWORDS: *Tamarindus indica seed, Methanol extract, Antimicrobial properties, Agar well diffusion, Broth microdilution*

INTRODUCTION

In recent years, the search for natural and sustainable antimicrobial agents has gained significant attention due to the emergence of antibiotic-resistant pathogens and the potential adverse effects associated with synthetic antimicrobial compounds. Herbal extracts have been traditionally used in various cultures for their therapeutic properties, including antimicrobial activity. Tamarindus indica, commonly known as tamarind, is a tropical tree whose seeds have been reported to possess a range of pharmacological activities, including antimicrobial properties.^[1]

The seeds of Tamarindus indica contain various bioactive compounds such as flavonoids, alkaloids, and phenolic compounds, which have been suggested to exhibit antimicrobial activity against a broad spectrum of microorganisms. Among the different extraction solvents, methanol has been widely used for extracting bioactive compounds from plant materials due to its ability to efficiently solubilize a diverse range of phytochemicals.^[2]

This study aims to formulate a herbal cream containing the methanol extract of Tamarindus indica seeds and evaluate its antimicrobial efficacy against selected microbial strains. The formulation of the herbal cream will involve optimizing the concentration of the methanol extract to ensure maximum antimicrobial activity while maintaining the stability and safety of the cream. The antimicrobial activity of the formulated cream will be assessed using standard microbiological methods, and the results will be compared with those of commercially available antimicrobial creams.^[3]



The findings of this study could contribute to the development of natural and sustainable alternatives to synthetic antimicrobial agents, potentially offering a safe and effective treatment option for various microbial infections.^[4]

Manufacturing and processing the cold cream for antimicrobial use

Ingredients and material

1. Tamarind seed extract
 - Collect tamarind seed of specified variety
 - Wash thoroughly to remove contaminants
 - Dry the tamarind seed and taturates make fine powder of tamarind seed
2. Petroleum ether :
 - Choose pharmaceutical grade petroleum ether
 - Measure the required quantity to help for the extraction process .
3. Water :
 - Use distilled water for consistency and to avoid impurities
 - Also use water for the extraction process

Laboratory Equipment

Reagent bottle , measuring cylinder , mortar pestle , ph meter , UV visible spectroscopy , whatman filter paper , maceration , percolation . etc .

Formulation process

1.Extraction of active compounds from tamarind seed :

- use a suitable solvents (e.g. Ethanol , water)

2. preparation of tamarind seed extract :

Procedure :

1. Air dried seeds (16.71gm) were finely ground and soaked in petroleum ether 5 ml per day at room temperature for 3 days
2. Add 5ml per day petroleum ether and water in the sample for 10days
3. After sample taturated and filtered out through the vaccum filter
4. Check the wavelength of the extract under the UV spectroscopy

3.Preparation of cold cream :

Procedure :

- Phase 1 :
melt the solid ingredients by heat and add all oils mixture.

- Phase 2 :
Dissolve borax in water with the help of heat

Add phase 1 into phase 2 with constant stirring to the wax and oil mixture continue process for 5 min .

Formula

SR. NO	Ingredients	QTY Taken (100 ml)	Category
1	Tamarind seed extract	4 ml	Antimicrobial
2	Beeswax	15 gm	Base
3	Borax	0.50 gm	Emulsifying agent
4	Tween 80	Q. S	Emulsifying agent
5	Mint oil	20 ml	Perfumery

**Uses:****Fig. Tamarind seed**

A herbal cream containing antimicrobial properties from the methanol extract of *Tamarindus indica* seeds can have various uses, including:

1. **Topical Antimicrobial Treatment:** The cream can be applied to the skin to treat various microbial infections such as bacterial and fungal skin infections, including acne, dermatitis, and athlete's foot.
2. **Wound Healing:** The antimicrobial properties of the cream can help prevent infections in wounds and promote faster healing.
3. **Skin Care:** The cream can be used as a part of daily skincare routine to maintain healthy skin, prevent microbial infections, and alleviate skin conditions like inflammation and itching.^[5]
4. **Foot Care:** Due to its antifungal properties, the cream can be beneficial for foot care, particularly in preventing and treating fungal infections such as athlete's foot.
5. **Hygiene:** The cream can be used as a hygiene product to inhibit the growth of harmful microbes on the skin, particularly in situations where hygiene is compromised.^[6]
6. **Natural Alternative:** For individuals preferring natural remedies, the herbal cream provides a natural alternative to synthetic antimicrobial creams.
7. **Supportive Care:** It can be used as a supportive care option in conjunction with other treatments for microbial infections, enhancing their effectiveness and providing additional benefits.^[7]

Before using the cream, it's advisable to perform a patch test to check for any allergic reactions or sensitivities. Additionally, consulting a healthcare professional for specific conditions or concerns is recommended.

Mechanism of action

The antimicrobial properties of *Tamarindus indica* seeds, specifically in the context of their methanol extract, can be attributed to several mechanisms of action:

1. **Disruption of Cell Membrane:** Compounds within the extract may disrupt the integrity of microbial cell membranes, leading to leakage of cellular contents and eventual cell death.
2. **This disrupts the essential functions of the microorganism, inhibiting its growth and proliferation.**
3. **Interference with Metabolic Pathways:** Active compounds in the extract may interfere with microbial metabolic pathways, disrupting processes vital for the survival and reproduction of microorganisms. This can include inhibition of enzyme activity or interference with essential cellular processes, leading to microbial inhibition.
4. **Damage to DNA/RNA:** Some components of the extract may induce damage to microbial nucleic acids, including DNA and RNA, thereby impairing replication, transcription, and translation processes. This leads to the inability of the microorganism to proliferate and survive.



5. Generation of Reactive Oxygen Species (ROS): Certain constituents of the extract may stimulate the generation of reactive oxygen species within microbial cells. ROS can cause oxidative damage to cellular components, including proteins, lipids, and nucleic acids, ultimately leading to cell death.
6. Modulation of Microbial Biofilms: The extract may interfere with the formation and stability of microbial biofilms, which are protective structures formed by microorganisms. By disrupting biofilm formation or enhancing biofilm dispersal, the extract can render microorganisms more susceptible to antimicrobial agents and immune responses.
7. Enhancement of Immune Response: Some compounds in the extract may possess immunomodulatory properties, stimulating the host immune system to recognize and eliminate microbial pathogens more effectively.

These mechanisms collectively contribute to the antimicrobial efficacy of the methanol extract of *Tamarindus indica* seeds, making it a promising candidate for use in herbal cream formulations targeting microbial infections.^[8]

Application

The application of a herbal cream containing the antimicrobial properties of the methanol extract of *Tamarindus indica* seeds involves the following steps:

1. Cleanse the Skin: Start by cleansing the skin thoroughly with a mild soap and water. Pat the skin dry with a clean towel before applying the cream.
2. Take a Small Amount: Dispense a small amount of the herbal cream onto your fingertips. You typically only need a pea-sized amount for a small area, but adjust according to the size of the affected area.
3. Apply Gently: Use your fingertips to gently spread the cream over the affected area. Massage it into the skin using circular motions until it is evenly distributed.^[9]
4. Cover if Necessary: If directed by a healthcare professional or if the condition requires it, cover the treated area with a sterile bandage or dressing to protect it and promote absorption of the cream.
5. Frequency of Application: Follow the recommended frequency of application provided with the cream. This could range from once daily to multiple times per day, depending on the severity of the condition.
6. Continue Use: Consistently apply the cream as directed, even if symptoms improve. Complete the full course of treatment unless otherwise instructed by a healthcare professional.
7. Monitor for Side Effects: Keep an eye out for any signs of skin irritation, allergic reactions, or other adverse effects. If any occur, discontinue use and consult a healthcare professional.
8. Store Properly: Store the herbal cream according to the instructions on the packaging. This typically involves keeping it in a cool, dry place away from direct sunlight and heat.

By following these steps, you can effectively apply the herbal cream to harness its antimicrobial properties for various skin conditions.^[10]

Pharmacology

In pharmacology, understanding the pharmacodynamics and pharmacokinetics of the methanol extract of *Tamarindus indica* seeds is crucial for assessing its efficacy, safety, and potential interactions. Here's a brief overview:

1. Pharmacodynamics: This involves studying how the methanol extract interacts with microbial targets to exert its antimicrobial effects.

Key aspects include:

- Mechanism of action: As discussed earlier, the extract may disrupt microbial cell membranes, interfere with metabolic pathways, induce DNA/RNA damage, generate reactive oxygen species, and modulate microbial biofilms.

- Spectrum of activity: Assessing which microorganisms are susceptible to the extract's antimicrobial effects, including bacteria, fungi, and other pathogens.

- Concentration-response relationships: Determining the concentration of the extract needed to inhibit microbial growth or kill pathogens effectively.

- Time-course effects: Understanding how quickly the extract acts and how long its effects last once applied or administered.

2. Pharmacokinetics: This involves studying the absorption, distribution, metabolism, and excretion (ADME) of the active compounds in the extract within the body. Key considerations include:

- Absorption: How the active compounds are absorbed into the bloodstream or through the skin upon topical application.

- Distribution: How the compounds are distributed to target sites within the body or the skin.

- Metabolism: How the compounds are metabolized by enzymes in the body, potentially affecting their bioavailability and activity.

- Excretion: How the compounds are eliminated from the body, whether through urine, feces, sweat, or other routes.^[11]

3. Safety and Toxicology: Evaluating the safety profile of the extract, including:

- Acute and chronic toxicity: Assessing the potential for adverse effects from short-term or long-term exposure to the extract.

- Skin irritation and sensitization: Determining whether the extract causes irritation or allergic reactions when applied topically.



- Systemic effects: Monitoring for any systemic effects or interactions with other medications or substances.
 - 4. Interactions: Investigating potential interactions between the extract and other drugs or herbal products, both pharmacodynamic and pharmacokinetic in nature.^[12]
 - 5. Dose Optimization: Determining the optimal dose and dosing regimen based on pharmacokinetic and pharmacodynamic data, balancing efficacy with safety considerations.
- Overall, a comprehensive understanding of the pharmacology of the methanol extract of *Tamarindus indica* seeds is essential for its effective and safe use in herbal cream formulations with antimicrobial properties.^[13]

Chemical Constituents

The methanol extract of *Tamarindus indica* seeds contains various chemical constituents that contribute to its antimicrobial properties. Some of the key constituents include:

1. Polyphenols: Tamarind seeds are rich in polyphenolic compounds such as flavonoids and phenolic acids. These compounds have antioxidant and antimicrobial properties and may contribute to the extract's effectiveness against microorganisms.
 2. Tannins: Tannins are polyphenolic compounds with astringent properties found in *Tamarindus indica* seeds. They have been reported to possess antimicrobial activity against a wide range of bacteria and fungi.
 3. Saponins: Tamarind seeds contain saponins, which are glycosides with surfactant properties. Saponins have been shown to exhibit antimicrobial activity by disrupting microbial cell membranes.
 4. Alkaloids: Some alkaloids have been identified in *Tamarindus indica* seeds. While their specific antimicrobial properties may vary, certain alkaloids have been reported to exhibit antibacterial and antifungal activities.^[14]
 5. Phytosterols: Phytosterols are plant-derived compounds structurally similar to cholesterol. They have been reported to possess antimicrobial properties and may contribute to the overall antimicrobial efficacy of the extract.
 6. Polysaccharides: Tamarind seeds contain polysaccharides such as galactoxyloglucan, which have been studied for their potential antimicrobial activities. These polysaccharides may help inhibit the growth of microorganisms by interfering with their cellular processes.
 7. Fatty Acids: Tamarind seeds contain fatty acids, including both saturated and unsaturated fatty acids. While fatty acids themselves may not exhibit direct antimicrobial activity, they can contribute to the overall composition and properties of the extract.^[15]
- These chemical constituents work synergistically to exert antimicrobial effects against a wide range of microorganisms, making the methanol extract of *Tamarindus indica* seeds a promising natural source for antimicrobial applications, including in herbal cream formulations.

Taxonomic Information

Tamarindus indica, commonly known as tamarind, belongs to the plant family Fabaceae, which is also known as the legume, pea, or bean family. Here's some taxonomic information about *Tamarindus indica*:

- **Kingdom:** Plantae
- **Phylum:** Angiosperms (flowering plants)
- **Class:** Eudicots
- **Order:** Fabales
- **Family:** Fabaceae
- **Genus:** *Tamarindus*
- **Species:** *Tamarindus indica*

Tamarindus indica is a tropical tree native to Africa but is cultivated and naturalized in various tropical regions around the world, including parts of Asia and the Americas. It is widely known for its edible fruit, which is used in culinary applications and traditional medicine. Additionally, various parts of the *Tamarindus indica* tree, including the seeds, bark, and leaves, are utilized for their medicinal and therapeutic properties.^[16]

Therapeutic

The methanol extract of *Tamarindus indica* seeds has several therapeutic applications, primarily due to its antimicrobial properties and other bioactive constituents. Some therapeutic uses include:

1. Antimicrobial Activity: The extract has demonstrated efficacy against various microorganisms, including bacteria, fungi, and viruses. It can be used topically to treat skin infections such as acne, dermatitis, fungal infections, and wounds.
2. Anti-inflammatory Effects: Certain constituents in the extract exhibit anti-inflammatory properties, which can help alleviate symptoms associated with inflammatory skin conditions like eczema, psoriasis, and allergic reactions.



3. **Wound Healing:** The antimicrobial and anti-inflammatory properties of the extract contribute to its ability to promote wound healing. It can help prevent infections in wounds and facilitate tissue repair and regeneration.
 4. **Antioxidant Activity:** Polyphenolic compounds in the extract possess antioxidant properties, scavenging free radicals and reducing oxidative stress. This can benefit overall skin health and may have anti-aging effects.^[17]
 5. **Immunomodulatory Effects:** Some constituents of the extract have been reported to modulate the immune response, potentially enhancing the body's ability to fight infections and promote healing.
 6. **Analgesic Effects:** The extract may have mild analgesic properties, providing relief from pain and discomfort associated with skin conditions or wounds.
 7. **Hydrating and Moisturizing:** Certain components of the extract can help hydrate and moisturize the skin, improving its overall texture and preventing dryness and irritation.
 8. **Skin Lightening:** Tamarindus indica seed extract has been investigated for its potential to inhibit melanin production, making it useful in cosmetic formulations aimed at reducing hyperpigmentation and promoting a more even skin tone.
 9. **UV Protection:** Some studies suggest that the extract may offer protection against UV-induced skin damage, although further research is needed to fully elucidate its photoprotective effects.
- These therapeutic properties make the methanol extract of Tamarindus indica seeds a valuable ingredient in herbal creams and skincare products targeting various skin conditions and promoting overall skin health. However, it's essential to consult a healthcare professional or dermatologist before using any herbal remedies, especially for chronic or severe skin conditions.^[18]

Traditional knowledge

Traditional knowledge regarding Tamarindus indica encompasses a wide range of uses and practices across different cultures. Here are some examples of traditional knowledge associated with tamarind:

1. **Culinary Use:** Tamarind is widely used as a culinary ingredient in many cuisines around the world. Its sour taste adds flavor to dishes such as curries, chutneys, sauces, soups, and beverages. In some cultures, tamarind is also used to make candies and snacks.
2. **Medicinal Use:** In traditional medicine systems such as Ayurveda, Traditional Chinese Medicine (TCM), and various indigenous healing practices, different parts of the tamarind tree, including the fruit, seeds, leaves, and bark, are used for their medicinal properties. Tamarind is believed to have digestive, laxative, anti-inflammatory, antimicrobial, and antioxidant properties. It is used to treat various ailments such as digestive disorders, fever, sore throat, skin conditions, and more.
3. **Oral Health:** In some cultures, tamarind is used for oral hygiene. Chewing on tamarind pulp or using tamarind-based toothpaste is believed to help maintain oral health, prevent gum disease, and freshen breath.^[19]
4. **Textile Industry:** Tamarind seeds contain a sticky pulp that can be used as a mordant in the textile industry to fix dyes to fabrics. Tamarind seed powder is also used as a sizing agent in textile sizing processes.
5. **Construction:** Tamarind wood is known for its durability and resistance to termites, making it suitable for construction purposes such as making furniture, doors, windows, and other wooden structures.
6. **Cultural and Religious Significance:** Tamarind trees are sometimes considered sacred and are planted near temples, shrines, and religious sites. The fruit and leaves may also have symbolic significance in certain cultural and religious ceremonies.^[20]
7. **Household Uses:** Tamarind pulp is used as a natural cleaning agent due to its acidic properties. It can be used to clean copper, brass, and other metal surfaces.
8. **Veterinary Medicine:** Tamarind extracts are sometimes used in traditional veterinary medicine to treat livestock ailments such as digestive disorders and skin infections.

Overall, tamarind has a rich history of traditional knowledge and diverse uses across various aspects of human life, including food, medicine, industry, and culture.^[21]

Quality Control

1. **pH measurement :**
 - Check the the pH of the formulation to ensure it is suitable for oral use .
2. **Stability testing :**
 - store samples in cool condition .
3. **Texture and consistency testing :**
 - Evaluate the texture and consistency of the cold cream to ensure a comfortable application .
4. **Antimicrobial activity study :**

In this study we check the antimicrobial activity using a agar medium plate formation method we check the microbial growth are increasing or decreasing .^[22]

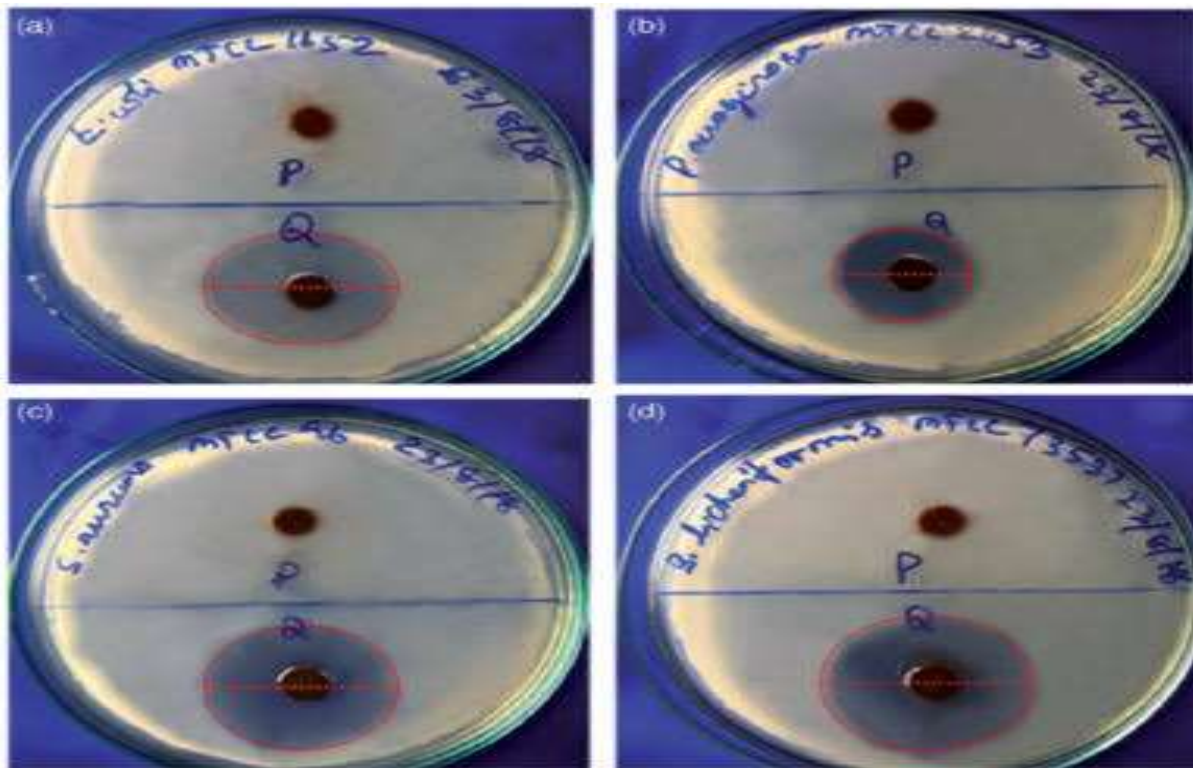


Fig : To check the antimicrobial activity using a different bacteria

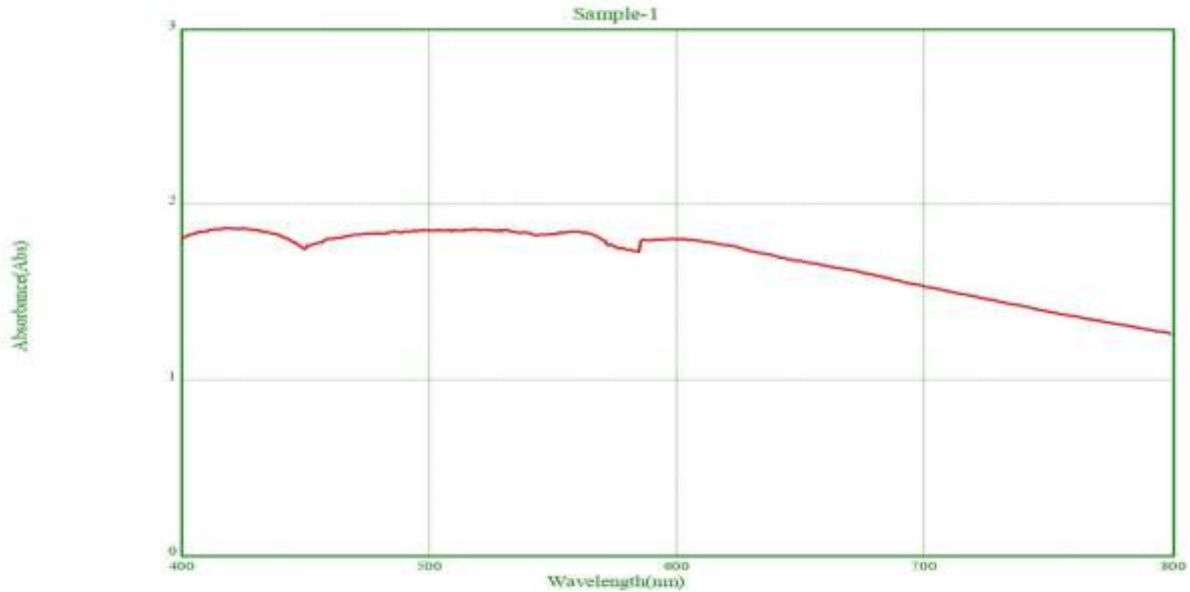
Result , Observation , And Evaluative steps

SR.NO	1) Physical Test	2) Sensory Test
1	Appearance - smooth texture	Odour – Refreshing odour Irritancy – No irritation and edema found
2	Colour – yellowish	
3	Texture – smooth	
4	Consistency – semi solid	
Sr . no	3) Chemical Test	4) Stability Testing
1	pH – 4 to 6.7	Temperature – Room temperature(25°C) and below Storage Duration – Stable for upto 12 months when stored in cool & dry place
2	Phenolic – present	
3	Flavonoids – Present	
4	Caffeic – Moderate	



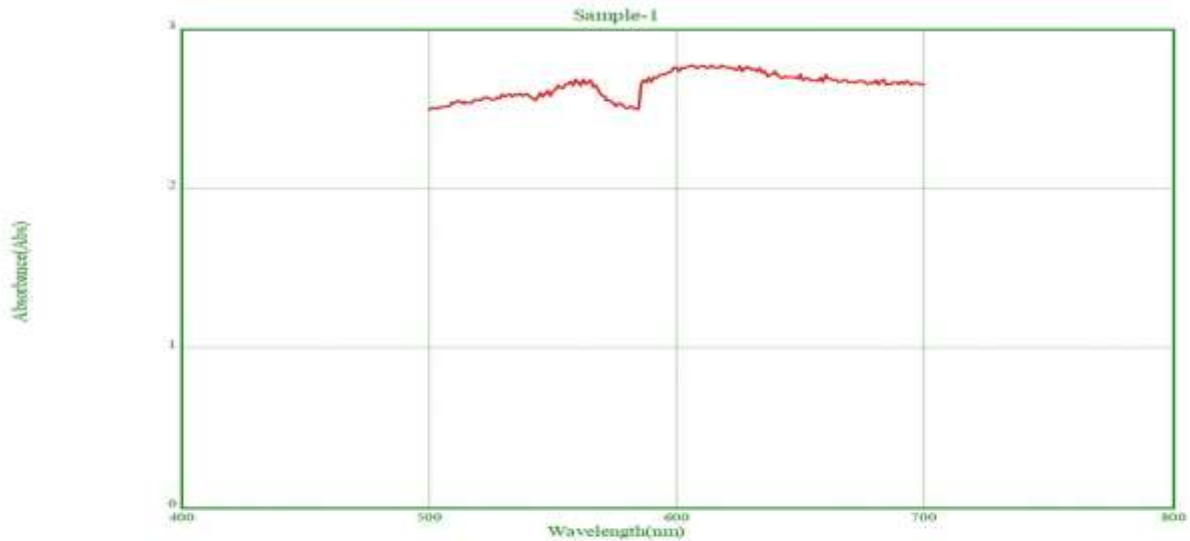
1. UV Frequency λ_{max} (Absorbance) Absorbance Vs Wavelength :

- Sample 1:



Scan Range: 400.0-800.0nm
Scan Step: 1.0nm
Scan Filter: 49
Scan Time: March 28 15:38:51 2024

- Sample2



Scan Range: 500.0-700.0nm
Scan Step: 1.0nm
Scan Filter: 49
Scan Time: March 21 15:14:04 2024

**• Conclusion on cold cream**

- In conclusion, *Tamarindus indica*, commonly known as tamarind, is a versatile plant with a wide range of uses and significance across different cultures and industries. Its edible fruit, medicinal properties, durable wood, and cultural symbolism make it an integral part of many societies around the world. From culinary applications to traditional medicine, textile production, and beyond, tamarind plays a multifaceted role in human life.
- Furthermore, ongoing research continues to uncover new insights into the biochemical composition and therapeutic potential of tamarind, particularly in areas such as antimicrobial activity, antioxidant effects, and oral health. This traditional knowledge, combined with modern scientific understanding, highlights the value of tamarind as a valuable resource for both traditional and contemporary applications.
- Overall, tamarind stands as a testament to the enduring relationship between humans and plants, demonstrating how traditional knowledge, cultural practices, and scientific advances

REFERENCE

1. Terefe Y., Deblais L., Ghanem M., Helmy Y.A., Mummied B., Chen D., Singh N., Ahyong V., Kalantar K., Yimer G., et al. Co-occurrence of *Campylobacter* species in children from eastern Ethiopia, and their association with environmental enteric dysfunction, diarrhea, and host microbiome. *Front. Public Health*. 2020;8:99. doi: 10.3389/fpubh.2020.00099.
2. Barbieri R., Coppo E., Marchese A., Daglia M., Sobarzo-Sánchez E., Nabavi S.F., Nabavi S.M. Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiol. Res*. 2017;196:44–68. doi: 10.1016/j.micres.2016.12.003.
3. Atwaa, E.S.H.; Shahein, M.R.; El-Sattar, E.S.A.; Hijazy, H.H.A.; Albrakati, A.; Elmahallawy, E.K. Bioactivity, Physicochemical and Sensory Properties of Probiotic Yoghurt Made from Whole Milk Powder Reconstituted in Aqueous Fennel Extract. *Fermentation* 2022, 8, 52.
4. Coman, V.; Teleky, B.-E.; Mitrea, L.; Martău, G.A.; Szabo, K.; Călinoiu, L.-F.; Vodnar, D.C. Chapter Five – Bioactive potential of fruit and vegetable wastes. In *Advances in Food and Nutrition Research*; Toldrá, F., Ed.; Academic Press: Cambridge, MA, USA, 2020; Volume 91, pp. 157–225.
5. Berrios R L, Arbiser J L. Effectiveness of gentian violet and similar products commonly used to treat pyodermas. *Dermatol Clin*. 2011;29:69–73.
6. Nigam PK, Syed HA, Saleh D. StatPearls [Internet]. StatPearls Publishing; Treasure Island (FL): Oct 29, 2023. Tinea Pedis.
7. Feng D., Zhang A., Yang Y., Yang P. Coumarin-containing hybrids and their antibacterial activities. *Arch. Pharm*. 2020;353:e1900380. doi: 10.1002/ardp.201900380.
8. Silva C.d.B.d., Guterres S.S., Weisheimer V., Schapoval E.E. Antifungal activity of the lemongrass oil and citral against *Candida* spp. *J. Braz. J. Infect. Dis*. 2008;12:63–66. doi: 10.1590/S1413-86702008000100014.
9. Pohorille A., Pratt L.R. Is Water the Universal Solvent for Life? *Orig. Life Evol. Biosph*. 2012;42:405–409. doi: 10.1007/s11084-012-9301-6.
10. Lewohl M, Clark L, Levitt J. Therapy for head lice based on life cycle, resistance, and safety considerations. *Pediatrics*. 2007 May;119(5):965-74.
11. Murugan K. Solanum alkaloids and their pharmaceutical roles: a review. *Journal of Analytical & Pharmaceutical Research*. 2015;3(6):1–14. doi: 10.15406/japlr.2016.03.00075.
12. Darbar S., Saha S., Pramanik K., Chattopadhyay A. Preliminary assessment of acute and 28-day repeated dose oral toxicity of a newly developed herbal mixture on experimental animal. *Indian J. Pharma. Edu. Res*. 2019;54(1):135–142. doi: 10.5530/ijper.54.1.16.
13. Schropp J, Khot A, Dhaval SK, Koch G. Target-mediated drug disposition model for bispecific antibodies: properties, approximation, and optimal dosing strategy. *CPT Pharmacomet. Syst. Pharmacol*. 2019;8(3):177–187. doi: 10.1002/psp4.12369.
14. Razali, N.; Mat Junit, S.; Ariffin, A.; Ramli, N.S.; Abdul Aziz, A. Polyphenols from the extract and fraction of *T. indica* seeds protected HepG2 cells against oxidative stress. *BMC Complementary Altern. Med*. 2015, 15, 438.
15. Altmann S.W., Davis H.R., Jr., Zhu L.J., Yao X., Hoos L.M., Tetzloff G., Iyer S.P., Maguire M., Golovko A., Zeng M., et al. Niemann-Pick C1 Like 1 Protein Is Critical for Intestinal Cholesterol Absorption. *Science*. 2004;303:1201–1204. doi: 10.1126/science.1093131.
16. Brandis D. 1906. *Indian Trees*. Bishen Singh Mahendra Pal Singh, Dehradun, India.
17. Feng D., Zhang A., Yang Y., Yang P. Coumarin-containing hybrids and their antibacterial activities. *Arch. Pharm*. 2020;353:e1900380. doi: 10.1002/ardp.201900380.
18. Jantan I., Ahmad W., Bukhari S.N. Plant-derived immunomodulators: An insight on their preclinical evaluation and clinical trials. *Front. Plant Sci*. 2015;6:655. doi: 10.3389/fpls.2015.00655.
19. Naureen, Z.; Medori, M.C.; Dhuli, K.; Donato, K.; Connelly, S.T.; Bellinato, F.; Gisondi, P.; Bertelli, M. Polyphenols and lactobacillus reuteri in oral health. *J. Prev. Med. Hyg*. 2022, 63 (Suppl. S3), E246–E254.
20. Framarin C. G. *The moral standing of animals and plants in the Manusmrti // Philosophy East and West*. 2014. V. 64. N. 1. P. 192–217.
21. Awada E, Austina D, Lyndona A, Awaad A. 2019. Possible effect of hala extract (*Pandanus tectorius*) on immune status, anti tumour and resistance to *Yersinia ruckeri* infection in rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immunol* 87: 620-629. DOI: 10.1016/j.fsi.2019.02.012.
22. Achkar, J.M.; Fries, B.C. *Candida* Infections of the Genitourinary Tract. *Clin. Microbiol. Rev*.